

# **SPECIMEN PREPARATION FOR ELECTRON MICROSCOPY**

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The purpose of electron microscopy is to study accurately the microstructures at high resolution and defect structure in a material. The knowledge of suitable sample preparation technique is essential for preparing a good representative sample of the material. The preparation techniques for SEM and TEM samples are different.

In general the SEM sample preparation techniques are more or less similar to the metallographic sample preparation technique for optical microscopy, because both the microscopes reveals the surface topography of sample. In SEM a focussed beam of electrons scans over the specimen surface and the electrons emitted from the sample surface controls the brightness of the CRT spot which also scans the CRT screen with the same raster as the scanning beam.

TEM sample has to be thin enough to allow the incident electron beam to pass through the specimen. Moreover the thin region of the specimen should be representative of the bulk material. The contrast in the image occurs due to the diffraction of the electron beam during transmission through the crystalline materials.

## **Sample preparation for SEM**

In general SEM samples can be classified into three categories : (a) Sample for fracture study, (b) Sample for microstructural study, (c) Powder samples for study of morphology i.e., shape and size of powder particles.

In all cases the samples are to be perfectly clean and all non-conductive samples must be coated with a thin layer of carbon or Au. Carbon coating is used when the chemical composition of the sample has to be examined by X-ray micro analysis.

For fractography the fractured sample is carefully cut according to the size that a specimen holder can accommodate, without any alteration of the fractured surface to be examined. It is then cleaned using some solvents, e.g. Acetone, Carbon tetrachloride, Alcohol to remove the dust, oil, grease or any extraneous materials. If the sample is non-conductor it is then coated using a vacuum evaporator or a sputter coater.

For study of microstructure a sample of suitable size is taken. It is ground and polished and then etched using suitable etchant. The procedure in this case is similar to that of optical metallography. A

stronger etch gives a better contrast in the image. The cleaned etched sample is ready for observation in the SEM.

For particle shape and size determination of powder sample, the powder must be distributed evenly over a clean substrate. A small quantity of powder is taken in a test tube filled with some solvent or, distilled water. It is then agitated in a ultrasonic cleaner to disperse the agglomerated particles. With the help of dropper, one or, two drops of the solvent is dropped on a clean glass slide. When the slide is dried a self adhesive conducting tape is pressed over the glass slide so that all the particles comes out of the slide when the tape is stripped out. The tape is then put on a mount and coated with Gold or carbon.

If the sample does not contain agglomerated particles, then a small quantity of powder is taken on cotton swab tip. The cotton swab tip is then held over either a conductive tape fixed on the top surface of the mount or top surface of the mount smeared with a drop of adhesive. The mount with adhesive is allowed to dry and then conductive coating of Gold or Carbon is given.

The powders can also be mixed with cold setting resins and the mixture is put inside a piece of polythene tube. After drying the polythene tube is separated from the solid resin sample by cutting. The sample is then ground, polished, cleaned and coated with Gold or Carbon before examination in SEM.

Insitu replicas are observed in SEM by cutting a suitable size of the replica and mounting it on a stub with its replicated side upwards. Gold coating is necessary before examination of replica in SEM.

### **TEM Sample Preparation**

In TEM, the high energy incident electron beam transmits through the sample, and while transmitting the beam gets diffracted where it encounters a defect such as grain boundary, the precipitate matrix interface, twin boundary, dislocation and stacking faults etc.

In order to reveal these crystal defects, the sample must be very thin, of the order of a few thousand Angstroms ( $1\text{\AA} = 10^{-10}$  metre). If the specimen is thick then most of the incident electrons will be absorbed in the sample and very less amount of electrons will come out from the bottom of the sample resulting in a poor quality image.

Another criteria most important during TEM sample preparation is the chance of introducing defects in the sample. So one has to be very careful to avoid such artefacts while preparing a TEM sample.

TEM samples can be classified into the following three categories (a) Thin films, (b) Thin foils, and (c) Replicas, among which thin foils and replicas are important in case of metallographic study.

Thin films of materials are prepared by Physical Vapour Deposition Technique. The material is kept in a Tungsten or Molybdenum basket or boat and a clean substrate is placed above or,

below the basket. The whole system (Fig.1) is evacuated to a vacuum of the order of  $10^{-5}$  m bar. The basket or boat is then heated by passing current through it. When the material melts it evaporates and gets deposited on substrate. A clean glass slide coated with uniform layer of soap or, NaCl can be used as substrate. When the thin film is formed on the slide, it is striped out by dipping the slide in distilled water. The soap or NaCl coating dissolves in water and the thin film floats on water surface. The floating films are fished out on EM grids, dried and then can be loaded in the TEM specimen holder. Thickness of of the films can be precisely monitored by Quartz crystal thickness monitor. However, thin film thickness can be worked out by the following simple calculations :

$$t = m / 4\pi r^2 \rho \text{ where } m = \text{mass of evaporant}$$

$$r = \text{distance of filament from substrate}$$

$$\rho = \text{density of evaporant}$$

$$t = \text{thickness of coating}$$

Other than physical vapour deposition, Electron beam evaporation, RF and DC Sputtering and Ion Beam sputtering are also used for making thin films.

Carbon support grids for TEM can also be made the same way provided carbon arcing system is fitted instead of a boat or, basket (Fig. The thickness of carbon film can be calculated as  $t = \frac{d^2 l}{16r^2}$  where  $l$  = length of carbon rod evaporated;  $d$  = diameter of carbon rod;  $r$  = distance of carbon tip from glass slide and  $t$  = thickness of coating.

Thin foil specimens are widely used for examining the metallography of samples by TEM. Initially thin slices ( $1\text{mm} > t > 0.5\text{mm}$ ) are cut using a low speed diamond saw with cooling arrangement. The use of low speed saws reduces the scruff loss and introduces minimum deformation in the sample. The thin slices are further thinned by either chemical dissolution or, mechanical hand grinding. Chemical dissolution reduces the thickness rapidly but chances of etching and pitting are there. Where as mechanical grinding produces a thin deformed layer on the sample which is subsequently removed during chemical thinning or electropolishing. When the thickness reaches nearly 0.1mm, the slice is cleaned thoroughly and subjected to final electropolishing.

In electropolishing technique the sample is made as anode dipped in a suitable electrolyte and the circuit is completed by putting platinum or, stainless steel cathode in it. When DC current is passed through the circuit, anodic dissolution takes place and the sample gets thinned down.

Electropolishing techniques used for making TEM samples are : (a) Window technique, (b) Bollmann technique, and (c) Jet thinning technique.

In window technique, a sample of roughly 2 cm length, 1 cm breadth and 0.1mm thick is taken and its edges are protected by

applying a protective lacquer (Lacomat) of width 1 mm to 2 mm on both sides. A simple electropolishing cell can be set up with a breaker, one pair of tweezers, two crocodile clips, suitable electrolyte, platinum or stainless steel cathode, and a variable voltage DC Power supply. After selection of proper voltage, the sample is connected to the circuit as anode and dipped into the electrolyte. Few minutes after switching on the DC supply, perforations in sample are visible near the top level of electrolyte, because of preferential polishing at the edge of the lacquered region. The sample is withdrawn from the electrolyte, circuit switched off and electropolishing started again with sample in inverted position. This time the dissolution of sample starts from both upper and lower edge. Ultimately when a narrow bridge between both sides of the sample is formed, the specimen is withdrawn from the electrolyte and circuit switch off. The typical stages during electropolishing are shown schematically in Fig.2. After washing the sample several times with alcohol, the central bridge portion containing the electron transparent area is cut with a sharp scalpel and sandwiched between two copper grids to observe in TEM.

The success of this technique in obtaining a uniformly thin sample without deformation depends upon the following factors :

- i) Proper electrolytic bath
- ii) Selection of proper voltage and current density,
- iii) Proper temperature of electrolyte,
- iv) Proper agitation of the bath
- v) Careful handling of the specimen.

A list of common electrolytic baths used for some metals and alloys are given in Table-1. Electrolytic baths and conditions for other materials are available in the literature and reference books.

A typical current-voltage relationship (Fig.3) shows that the plateau region BC of curve ABCD results in good electropolishing. The curve EF shows the absence of plateau at higher temperature of the bath.

Cathodes of several configurations are used, but a stainless steel sheet or, wire gauze bent in the form of a hollow cylinder gives good result.

In the Bollmann technique, two pointed cathodes with sharp ends pointing towards the sample are placed on both sides of the sample (Fig.4). Initially a small hole is formed in the sample near the cathode tips. The specimen is then shifted a little to form another hole in sample. In this way several perforations at very small gap are formed in the sample. Finally the bridge portions between holes are cut and washed in the same way as in window technique.

Twin jet electropolishing is widely used for making TEM samples from metals and alloys for the following advantages : (i) Several 3 mm disc samples can be prepared from a preliminary thinned foil (thickness ~ 0.1mm), (ii) The jet action from both sides helps in reducing the deformation of thin area and removes reaction products



normally adhering to the thin foil surface, (iii) Final thinned disc can be directly mounted on the specimen holder without using any grid, (iv) Handling of thin discs are more convenient.

In this technique 3 mm dia discs are punched from a preliminary thinned foil of thickness nearly 0.1mm. One disc is fitted in the anode holder and placed in between two cathodic jets such that the jets hit the centre of the disc (Fig.5). Due to the action of two cathodic jets, the sample disc (anode) thins down and forms a double concave type contour in the central region. Thinning is continued till a small perforation is formed in the centre of the sample disc to allow the light rays to pass through it and activate a photo cell. The small perforation is also detected by IR source and IR detector in case of coloured or turbid electrolytes. The sample is then removed from anode holder and washed several times carefully with alcohol. The washed and dried disc can be directly mounted on the sample holder. In the region near the edges of the central hole, electron transparent areas are observed in TEM. Similar to Window or Bollmann technique, the electrolyte composition, the temperature of the electrolyte and selection of proper DC voltage plays important roles in achieving success by twin jet technique. The other factors to be taken care are control of electrolyte pump flow rate and adjustment of detector sensitivity. The electrolytes used in this technique are similar to those used in window method. The voltage is selected either from literature or by trial and error method. Once the parameters are fixed several samples can be prepared within very short time.

Ceramic non-conductor samples can not be thinned by any of the above electropolishing techniques. Such samples are thinned by Ion Beam Thinning Technique. A beam of 3 -6 Kv argon ions are directed at a shallow angle ( $15^{\circ}$ - $30^{\circ}$ ) towards the sample (Fig.6). Atoms and molecules of the sample are ejected by the impingement of the ions. Argon ions are used because it is the easily available heaviest inert atom. The rate of thinning by this technique is slow and depends upon the following factors, (i) the ion energy, (ii) the relative masses of ion and specimen atom, (iii) the crystal structure of the specimen, (iv) the angle of incidence of the ion beam (Fig.7). Because of the slow thinning rate (0.5 - 0.15 $\mu$ m/hr), the samples are normally dished prior to ion beam thinning. Ultrasonic disc cutter produces 3 mm dia disc from a thin (0.5 to 1mm thickness) slice of brittle refractories. The disc is subjected to dimple grinding in which a small grinding wheel (~1 cm dia) rotates in a vertical plane whereas the sample rotates in horizontal plane resulting in a concave surface of the disc. When such dimpled disc is subjected to ion beam thinning the centre of the sample is first to form a hole which is detected by a laser detector. Similar to disc metal samples, the thin (electron transparent) areas are found in the regions near the hole. Ion beam thinning for short time also helps in cleaning the electropolished metal disc samples.

**Replica :** Since scanning electron microscopy is convenient to study the surface topography, replicas are not generally used for the same purpose. Replicas are used mainly for : (i) Insitu-metallography, (ii) Materials susceptible to electron beam damage (e.g. polymer), (iii) Accurate characterisation of precipitates particularly by electron

diffraction, X-ray micro analysis without interference from matrix, and (iv) very high resolution surface study.

Replicas are mainly of three categories : (i) Single stage, a negative replica, (ii) Double stage or, positive replica and (iii) Extraction replica.

Single stage replicas are prepared by pressing a cellulose-acetate film on the fractured or polished and etched surface, moistened with acetone or, chloroform and allowing the film to dry. The film is then peeled off from the surface. Carbon or carbon-platinum can also be deposited on the surface in vacuum evaporator. The film is then stripped from the surface by either chemical etching or, electro-chemical etching. To increase the contrast of image the replica is shadowed with gold or gold-palladium by evaporating at a shallow angle.

Double stage replicas are made by first making a single-stage plastic replica as described, and then depositing carbon on the side of plastic replica containing the structures. The plastic is then removed by dissolving in acetone or chloroform. The carbon replicas floating on the surface of the solvent are then picked on grids and dried. The different stages of two stage replica are shown in Fig.8.

The sequence of operation of making extraction replicas are shown in Fig.8. The metal is first polished and etched to expose the precipitate particles. Carbon is deposited on the etched surface. The carbon coating is then scratched in grids with sharp scalpel. The specimen is then second time etched by dipping it in a stronger etchant. Due to action of etchant the matrix gets dissolved and the carbon replicas float on the surface of etchant. The replicas are then transferred to cleaning bath containing alcohol, with the help of replica pick up loops. Finally these are picked up on TEM grids, dried and are ready to be viewed in TEM.

Other methods of sample preparation includes making powder of brittle materials in clean agate mortar and pestle, and sprinkling the powder on formvar or, carbon coated grids. If the powder particle have tendency to agglomerate, it is dispersed in a solvent or distilled water and a drop is put over the formvar or carbon coated grid. It is always safe to coat the powder containing grid with carbon again in vacuum evaporator. Samples of layered structure materials (e.g. graphite) can be made by cleaving several times with the help of scotch tapes. Thin samples of glass can be prepared by blowing from molten condition.

There are several other methods for preparing electron microscopy samples, from biological samples which are not described here.

# REFERENCE:

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Table - 1

Material	Electrolyte	Temperature	DC Voltage
1. Aluminium and Aluminium Alloys	20 cc Perchloric Acid 80 cc Ethanol	<0°C	12-17
2. Copper and Copper Alloys	33 cc Nitric Acid 67 cc Methanol	< -30°C	8-16
3. Iron, Steel and Stainless Steel	10 cc Perchloric Acid 90 cc Acetic Acid	~10°C	10-25
4. Titanium and Titanium Alloys	6 cc Perchloric acid 35 cc n-Butanol 60 cc Ethanol	<-25°C	11-20

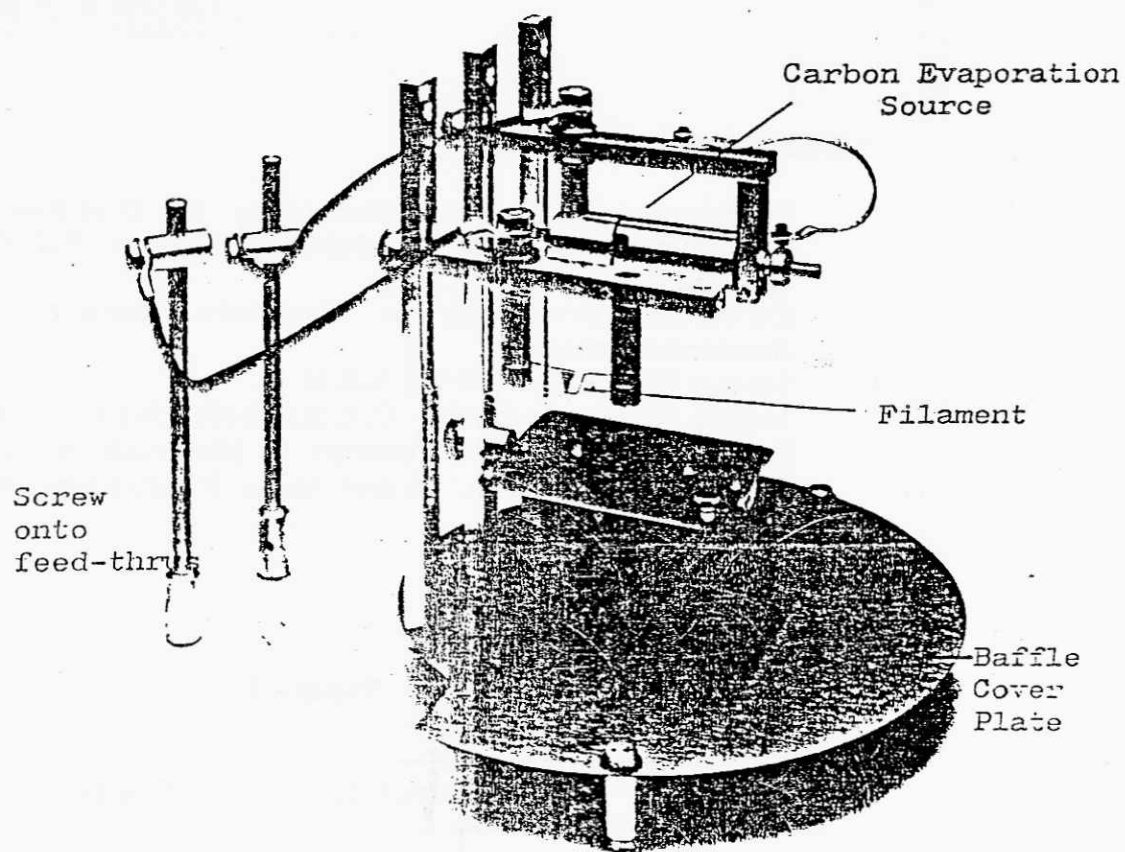


Fig.1: Jigging for Vacuum System

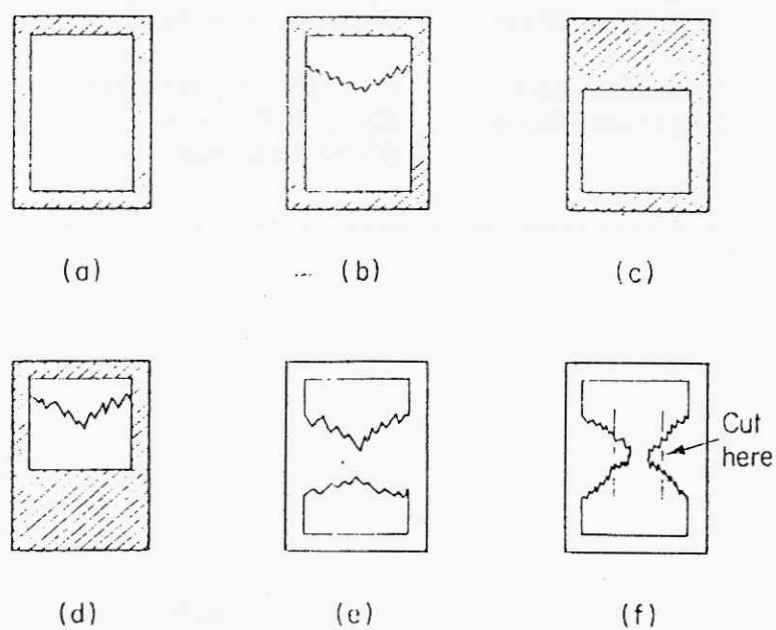


Fig.2 : Different stages in electropolishing using window technique.  
The shaded regions are lacquered



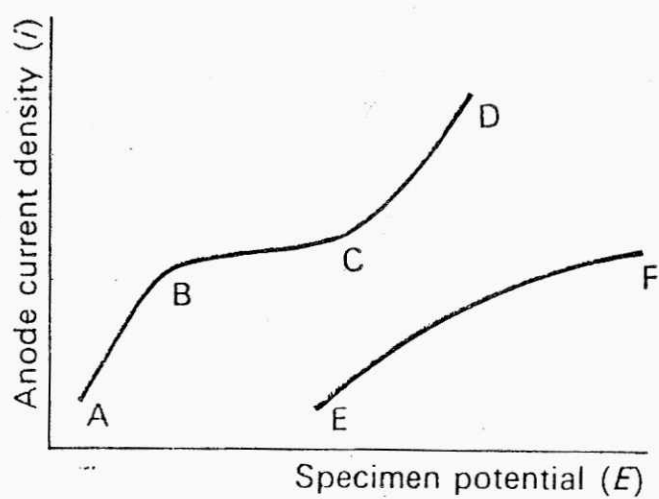


Fig.3 : Current voltage relationship during electropolishing

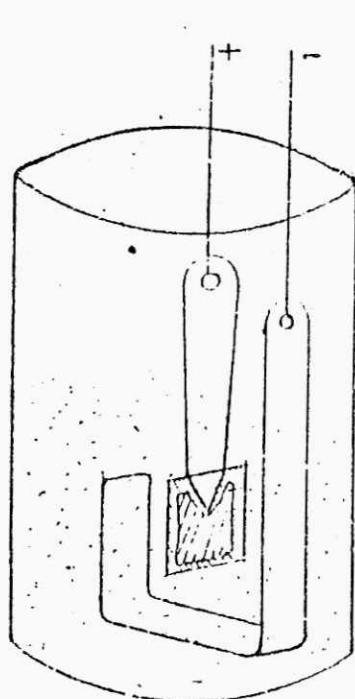


Fig A

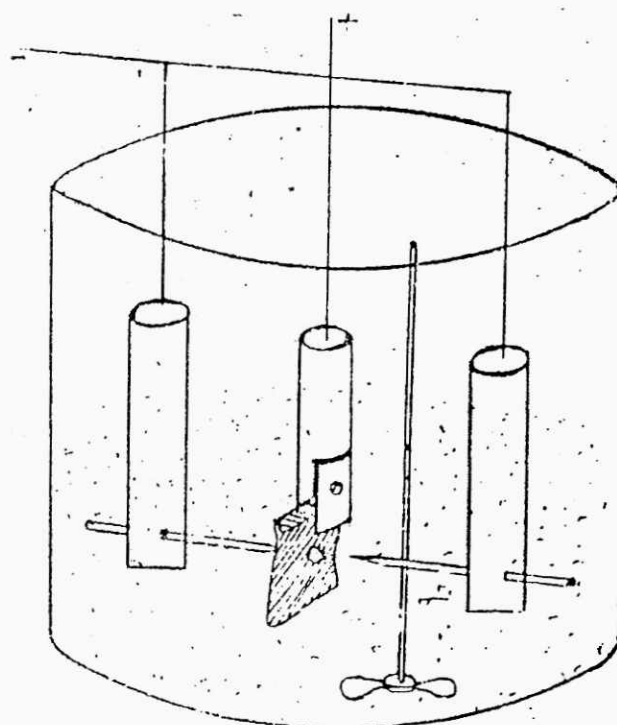


Fig B

Fig.4 : Electropolishing Methods (A) Window Technique  
(B) Bollmann Technique

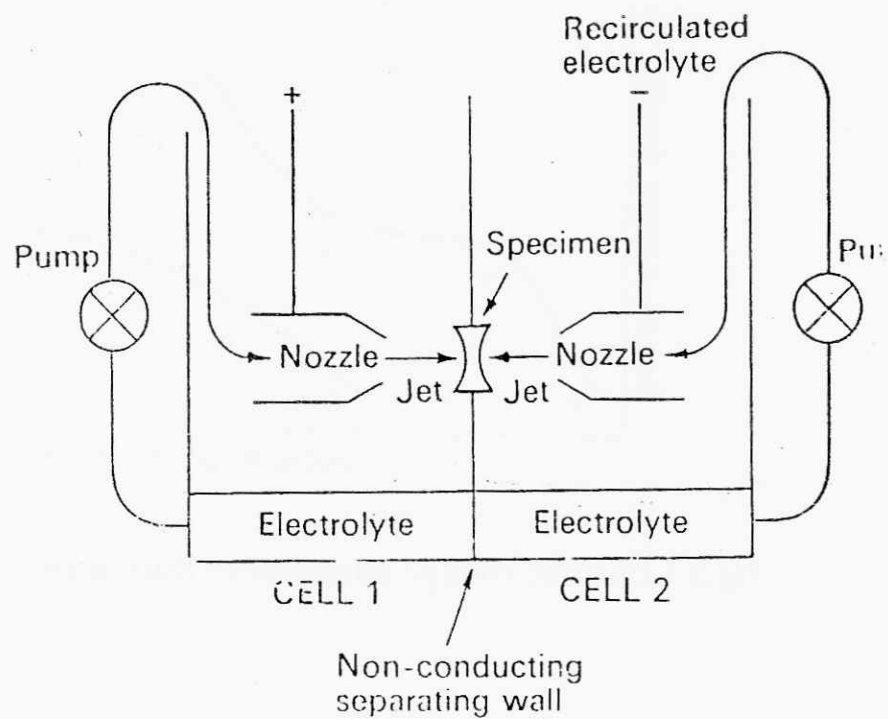


Fig.5: Schematic diagram of Twin Jet Technique

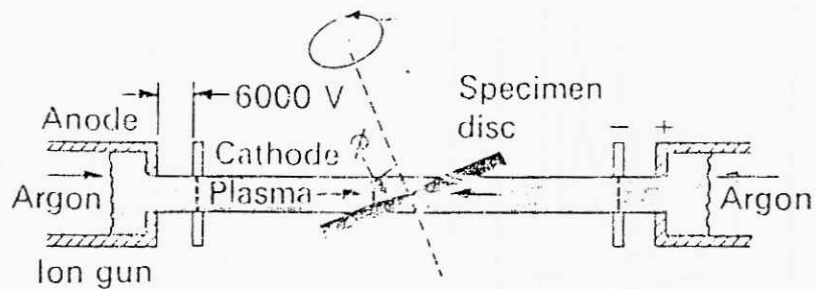


Fig.6 : Schematic showing Ion Beam Thinning Equipment

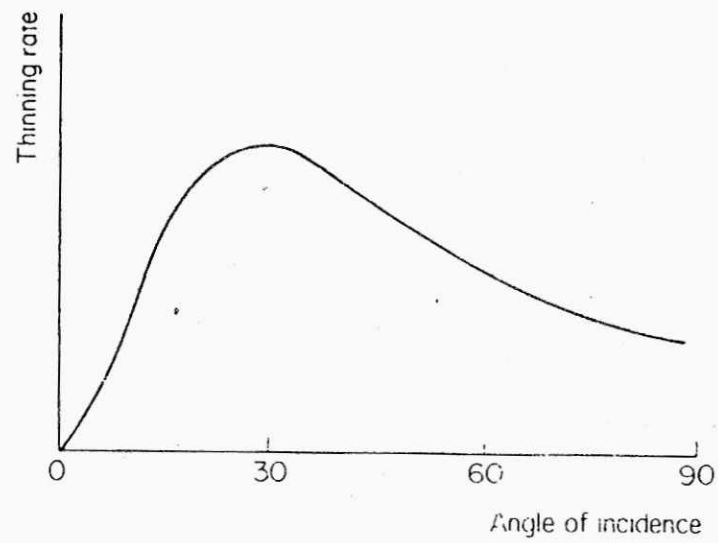


Fig.7 : Variation of Thinning Rate with Angle of Incidence during Ion Beam Thinning

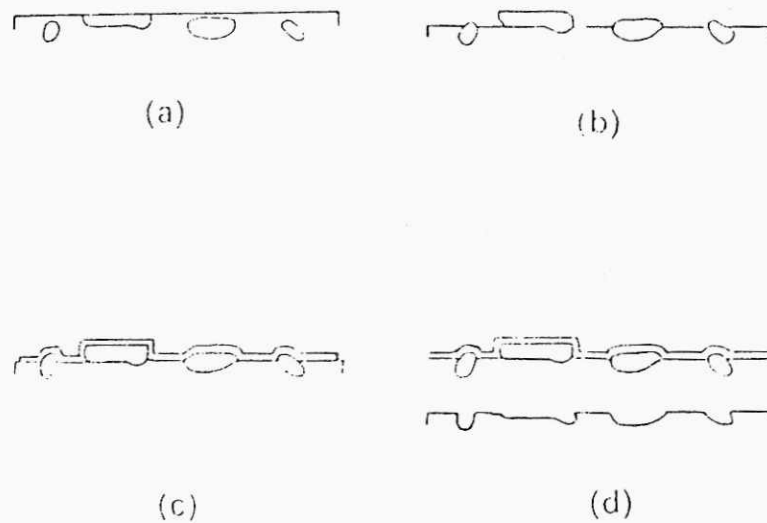


Fig. 8: Sequence of operations during preparation of Extraction Replica